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Breeding for Resistance to Wheat Streak Mosaic Virus

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BREEDING FOR RESISTANCE TO WHEAT STREAK MOSAIC VIRUS

BY

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BREEDING FOR RESISTANCE TO WHEAT

STREAK MOSAIC VIRUS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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SUMMARY

Immunity to WSMV was found in a Triticum Agropyron derived from Carsten x A. intermedium. This line was crossed with many common wheats and the F_1 seed irradiated. After one backcross to common wheat and several generations of selfing it was concluded that a desirable translocation had not occurred. A backcrossing program was began in an attempt to establish an addition line. After two backcrosses of resistant plants to common wheat one disomic addition plant and three monosomic addition plants were identified. A fifth resistant plant with 42 chromosomes was also identified. This plant apparently involves a translocation but it is unstable cytologically. Methods are described by which a translocation could be induced and thus transfer the immunity to WSMV into common wheat.

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INTRODUCTION

Wheat is one of the major cash crops in South Dakota producing annually about 36,600,000 bushels on approximately 2,050,000 acres. Many factors reduce the average yield to 17 to 18 bushels. Among them are drought, winterkill, hail, and disease. Plant breeders at South Dakota State University have, for the most part, been occupied with breeding for disease resistance, the most important contribution having been the movement of stem rust resistance from emmer into common wheat by McFadden. This one act has added immeasurable income to South Dakota and in fact most of the wheat producing regions of the world.

Next to leaf and stem rust, wheat streak mosaic virus (WSMV) is the most immediate disease threat to the South Dakota wheat crop. The first report of WSMV in South Dakota was in June of 1949, although there was indication of its being present earlier (33). In 1951 mosaic was found in Gregory, Tripp and Bennett counties. Although WSMV is a disease of winter wheat it can be severe on spring wheat grown adjacent to infected winter wheat. Infected plants are rarely killed but they can be affected in such a way as to reduce or prevent seed production. Many times a field is not worth harvesting. In recent years, estimates of the average reduction in yield due to the virus have been about 2 to 5% with some isolated areas suffering losses up to 20% (L. S. Wood, personal communication). No other estimates of economic losses in South Dakota have been attempted. To date the most severe outbreak of the disease was in Kansas in 1949 with a loss estimated at 30,000,000 dollars and in 1954 when an estimated loss of 14,000,000 dollars occurred.

Western Nebraska suffered an economic loss attributed to WSMV in 1962 of 500,000 dollars and in 1963 of 10,000,000 dollars (21).

The most successful method of controlling WSMV in winter wheat has been to delay seeding (17, 34). Results from the South Central substation near Presho, South Dakota, indicate that a delay in planting until after September 10 will effectively control the disease. It seems that the later planted wheat is able to escape infestation of the mite vector. The grower, however, does not base his planting date entirely on the threat of WSMV. He also considers soil moisture, amount of fall growth necessary to reduce erosion, number of acres to be seeded in the time available for planting and any increase in income which might come from fall grazing. For these reasons it is known that growers desire earlier planting dates.

In an extensive survey of the common wheats, no effective degree of resistance to WSMV has been found, although there did exist degrees of susceptibility (2, 3, 15, 29, 32). Some selections from certain intergeneric crosses involving Agropyron sp. have shown resistance (3). The purpose of this study was to transfer the resistance to WSMV from a Triticum-Agropyron selection into wheat.

REVIEW OF LITERATURE

The search for resistance to WSMV has been exhaustive. Bellingham, Fellows and Sill (3) summarized 7 years work that involved 2477 foreign winter wheat introductions, 1965 foreign and domestic spring wheats, 99 domestic winter wheats, and many selections from crosses between foreign and domestic varieties. Reactions to WSMV were usually extreme systemic susceptibility. Some tolerance was present in a few domestic winter wheats and in eleven foreign varieties but varied with environment, time of inoculation and other factors. Minimum losses in the tolerant varieties varied from 7 to 20%. Approximately 2400 plant selections from several tolerant varieties were made in an attempt to improve tolerance, all without success. A spray inoculation technique similar to that discribed by McKinney and Fellows (14) was used.

Andrews and Slykhuis (2) tested 1124 common wheat varieties against the natural vector wheat-leaf curl mite (Aceria tulipae, Keifer) and found that most varieties developed more severe symptoms than did the check variety, Kharkof. Sill, Bellingham and Fellows (32) tested 2000 spring wheat selections and varieties against the Salina strain of WSMV. After 3 years testing, 76 of the better ones were sent to Wyoming for further testing. Sill questioned whether the resistance would be of practical value. Sill et al. (32) tested an additional 2,433 entries from the world collection of common wheat and found all to be systemically invaded by the virus. There did appear to be some tolerance. Bohnenblust and Kolp (4) isolated what

seems to be the most tolerant common wheat, CI 13000, a soft white spring type. They concluded that CI 13000 had one set of genes for tolerance not present in Bison, a very susceptible winter wheat.

McNeal and Carroll (15) evaluated 12 varieties of spring wheat from one naturally infected yield test nursery. There was up to a 8.4% loss in test weight and a 24.4 to 54.5% reduction in yield. The greatest reduction in test weight was 8.4% for Centana. Fortuna showed a gain of 2.8% in test weight. Centana had the greatest yield reduction with a 54.4% loss and Chris the least with a 24.4% loss. They concluded that Wells, Crim, Fortuna, and Chris were the most tolerant spring varieties in their test.

Fellows and Schmidt (10), Schmidt, Sill and Fellows (26), and Sebesta and Bellingham (29) concluded that there did not exist a high degree of tolerance to WSMV among the common wheat varieties. Sebesta and Bellingham (29) suggested that a higher type of resistance could be provided by certain related genera.

Bellingham et al. (3) tested some rye by wheat and Agropyron by wheat derivatives and found that a few of them were resistant to WSMV but was doubtful if any of these lines were of agronomic value. Sill et al. (32) found resistance or hypersensitivity in the progeny of a few grass-like Agropyron by wheat crosses and in some rye by wheat crosses. Andrews and Slykhuis (2) tested 41 different Triticum by Agropyron derivatives and found that they differed considerably in their reaction to WSMV and to the mite, Aceria tulipae. His work suggested that possible sources of resistance were Agropyron elongatum, and

A. intermedium. Fellows and Schmidt (10) tested some Agrotriticum hybrids and found a wide diversity of reactions to WSMV. The more grass-like selections had the greatest amount of resistance. The Agropyron's used were A. elongatum, A. trichophorum, and Agropyron sp. (Sando). Check varieties indicated that there were some escapes. Shannor and Bridgmon (30) reported that eight different strains of intermediate wheatgrass were resistant to WSMV.

Schmidt et al. (26) tested certain advanced generation wheat x A. elongatum hybrids. Eighteen plants of each line tested ranged in reaction to WSMV from immunity to symptomless carriers to ultra susceptibility. Extensive tests were made of plants which gave a local lesion type reaction. Selection 52-6605 was such a hybrid and the F_1 between it and Pawnee gave a similar type reaction. Swarup et al. (35) found that 42 F_1 plants between the advanced Agroptriticum hybrid (2n=56) and Pawnee gave a local lesion reaction. The F_2 plants showed systemic, local lesion, and local lesion turning systemic type reactions. The segregations fit no apparent genetic ratios. F_3 lines segregated as did the F_2 populations. Chromosome numbers of the F_1's varied from 46 to 50 with an average of 48.16. There were 7.0 to 10.4 univalents per cell with an average of 8.47 in the F_1's tested.

In F_2, the plants with a local lesion reaction to WSMV had a significantly higher average chromosome number (49.5) than did plants which had a local lesion turning systemic reaction (45.6) or plants with a systemic reaction (43.6). The number of pollen quartets from the F_1 plants with micronuceli varied from 2 to 12 with an

average of 8.66. Lack of chromosome homology, unfavorable chromosome combinations, and genetic factors influencing pairing were suggested as possible causes of the variable meiotic index. Plants which gave the local lesion type reaction were more grass-like than plants with the other types of reactions. They concluded that the genes for resistance to WSMV in the particular Agrotriticums used were located on more than one chromosome and that transfer of these genes would be difficult or impossible. The use of other Agropyron species as sources of resistance was suggested.

Sebesta and Bellingham (29) using the complex Sando hybrid, Triticum sp. x A. elongatum (Host) Beau x [(Arlando x T. timopheevi Zhukou) x (Hope x Baart)] x Nebred, obtained a line P_3-19, which developed mild local lesion symptoms when inoculated with WSMV. Attempts to transmit the virus from it failed. F_1 plants from P_3-19 by Wichita gave a mild systemic reaction which was considered intermediate between the resistant and susceptible parent. F_2 plants segregated 4 resistant, 86 tolerant, 23 intermediate to tolerant, 50 intermediate, and 24 susceptible. It was suggested that this ratio for reaction to WSMV was the result of random chromosome segregation. F_3 plants from the 4 resistant F_2 plants segregated 33 resistant, 9 tolerant, and 50 susceptible when tested against WSMV. Cytological examination in F_3 of certain F_2 plants indicated that resistance diminished with the loss of Agropyron chromosomes. Exceptions to this trend were lines 5508 and 5522 apparently with 21 pairs of wheat chromosomes and 1 pair of Agropyron chromosomes carrying resistance to WSMV.

Sebesta proposed that the resistance be transferred by using irradiation to induce a translocation.

Raj (22) tested 35 advanced generation Agrotriticum hybrids against WSMV. These hybrids fell into 2 groups, (Chinese^2 - A. elongatum x Pawnee) x (Triumph x Kawvale - Marquillo - Tenmarq) and (wheat - A. elongatum) x Pawnee. Reaction classes were highly resistant, resistant, moderately susceptible, and susceptible. Of the 35 hybrids, 5 were grass-like and resistant, the remainder being wheat-like and varying in degrees of resistance. Six promising resistant lines were selected and crossed with Kaw, Wichita, Ottawa, and CI 13285 (Parker). Almost all the hybrids of crosses between wheat-like lines and commercial varieties had 42 chromosomes. Pairing at metaphase I in all but one of these hybrids was irregular. One hybrid had very good pairing with progeny segregating in a dihybrid ratio for reaction to WSMV. The transfer of the resistance was apparently due to a spontaneous translocation. Although selections were being made from F_2 populations, no further report or distribution of this material to wheat breeders has been made.

It is apparent that the best sources of resistance to WSMV are in the Agropyron's and rye. Unrau (36), in an extensive review of published work on wheat by Agropyron crosses, thought it highly improbable that Agropyron characteristics could be transferred to wheat through normal gene exchange because of the failure of pairing and crossing-over between wheat and Agropyron chromosomes. This problem may be circumvented by a method developed by Sears (27)

who utilized a method developed in 1940 by O'Mara (19) for the production of addition lines involving alien chromosomes. Sears suggested producing an amphiploid, backcrossing to wheat, and selecting from among the progeny those plants having the complete wheat complement plus an alien chromosome carrying the desirable gene. This has become the basic method for the production of addition lines in common wheat. Sears (27) crossed Triticum dicoccoides with T. umbellulata, which is resistant to leaf rust. He then produced a fertile amphiploid which was crossed with Chinese Spring. Pollen from the resulting plants was used on Chinese Spring. Forty-seven seeds were obtained and the resulting plants tested against leaf rust and scored cytologically. A plant which had 21 pairs and 3 univalents was crossed as male to Chinese Spring. There were 36 offspring, 5 of which were resistant to leaf rust. One of these 5 plants had 21 pairs of wheat chromosomes plus one T. umbellulata chromosome carrying rust resistance. It was selfed and 119 seeds obtained. Thirty of these plants were resistant and 28 had the single T. umbellulata monosome. These plants were irradiated about the time of meiosis with x-rays, and pollen from the irradiated plants used on Chinese Spring. The transmission of the monosome through the pollen prior to irradiation was low. By using pollen from irradiated plants it was thought that selective pressure would be applied in favor of an intercalary translocation. If an intercalary translocation involved a small enough piece of chromatin, it would be transmitted through the pollen normally. It was thought that an intercalary translocation had occurred but on

two occasions since Sears first published his work, Kimber (11) and Sears (28) indicated that the translocation was terminal. A breeders variety "Transfer" involving this translocation has been released.

Elliott (9), Knott (12) and Sharma and Knott (31) moved stem rust resistance from Agropyron into wheat. Wienhues (37) transferred leaf rust resistance from Agropyron into wheat. Bravo (5) translocated leaf rust resistance from rye to wheat. Acosta (1) moved stem rust resistance from rye into wheat. Sears (28) moved the hairy neck characteristic from rye into wheat. All translocations involved essentially the creation of a monosomic addition line, irradiation of plants Acosta (1) Sears (28), irradiation of seeds Sears (28), irradiation of seeds or plants Sharma and Knott (31) Wienhues (37), or of seeds Knott (12) Bravo (5). When plants were treated, pollen from the treated plants was used on normal wheat. When seeds were irradiated, the resulting plants were either selfed or used as pollen parents on normal wheat. Plants were treated with x-rays and seeds were treated with either cobalt 60 or fast neutrons. In either case the progeny were screened for the phenotypic effect sought from the alien chromosome. Cytological observations were made and those plants with the alien chromosome present as an addition were discarded. The remaining group was examined to detect the kind of transfer which involved the critical alien segment.

Driscoll and Jensen (6) described a method whereby the cytological examinations were limited to a smaller number of plants. Seeds from plants carrying the disomic addition of alien chromosomes, which

usually bred true, were irradiated and the resulting plants selfed. An induced translocation between one member of the rye chromosome pair and a wheat chromosome could cause meiotic irregularities leading to the loss of the resistance gene and segregation for susceptible plants in the progenies of the treated generation. Three-fourths of the R_2 head progenies were discarded since they did not segregate. Further tests of segregating rows led to the isolation of two lines which possibly contained the desired translocation. These lines, which had normal male transmission rates, were then crossed with Chinese Spring and the F_1's were examined for rust reaction and cytological normality. It appeared that a translocation had occurred. Sears (28) feels that more work needs to be done on this method. Considerable cytological work may ultimately be required to prove that a translocation had occurred.

MATERIALS AND METHODS

A collection of Triticum-Agropyrons, Table 1, was given to George Buchenau in the spring of 1963 for testing against WSMV. The hand-rub method of inoculation was used. L691, the most resistant of all, was selected as the parental line to be used as a source of resistance to WSMV. This line came from Lethbridge, Alberta, in 1962 as TA25. M. N. Grant had received it under the designation L691 from B. C. Jenkins who found it in Germany. L691 is a cross between Carsten and A. intermedium, and has a chromosome number of 56 (A. Wienhues, personal communication). L691 has been given the designation SDI 6415 and will be referred to as such.

Many crosses were made between SDI 6415 and common wheat in the summer of 1964. Most of the common wheat parents used were of spring type because SDI 6415 was so late in heading that little pollen was available from winter wheat. F_1 seed from the crosses was sent to Oak Ridge for irradiation. The seed was divided and two doses used, 2500 rads and 1000 rads of fast neutrons. The heavier dose destroyed all the treated seed (Table 2). Plants from seed treated with 1000 rads were highly sterile and so were used as the female parent in backcrossing to common wheat. The treated F_1's headed over a long period so any common wheat, winter or spring, was used as male. Offspring were subsequently either backcrossed again or selfed three or four generations while selecting for resistant wheat-like plants. Resistant plants were then backcrossed to wheat twice and chromosome counts made using the Feulgen method similar

to that of Ostergen and Heneen (20). The meiotic index was determined by counting the number of spores without micronuceli and dividing by the total counted.

The rub method of inoculation using 600 mesh carborundum as an abrasive was used during the early phases of the study. This method was later changed to a spray method (J. B. Tunac, personal communication). Twenty-five grams of fresh plant material were collected from plants showing WSMV symptoms and ground in a Hobart grinder. The juice and pulp were collected separately. The pulp was mixed with 40 ml of deionized water and passed through the grinder again. This process was repeated until there were 160 ml of diluted juice. Carborundum of 400 or 600 mesh or celite was used as an abrasive. The mixture was then placed in a "Port-a-Blast" portable sand blaster and sprayed on the plants at 60 to 70 psi with the end of the nozzle held about 15 cm from the plants. The opening through which the juice was drawn was reduced to 1-2 mm and extended to within 3 mm of the bottom of the container (W. S. Gardner, personal communication). Plants were inoculated in the 2-3 leaf stage.

In order to reduce the chance of mite contamination of greenhouse plants from the infected plants, especially when field collected inoculum was used, inoculum was centrifuged at 10,000 g. for 15 minutes. This spun out all the plant debris and mite eggs to the bottom of the centrifuge tubes. The supernatant was carefully decanted and used as inoculum.

Tissue for electron microscopy was cut into sections 1 mm by

6 mm under 5% glutaraldehyde in 0.09 M potassium phosphate buffer (pH 7) and fixed in the same solution for 4 hours. The tissue was then washed in cold buffer and transferred to cold 1% OsO_4 in 0.1 M potassium phosphate buffer (pH 7) and held there for 3 to 16 hours.

The complete imbedding mixture (J. Pengborn, personal communication) was Araldite 6005, Epon 812 and DMP-30 (tridimethylaminomethyl phenol). The plastic was mixed thoroughly before using and frozen in glass vials. Prior to imbedding of the tissue, the plastic was warmed to room temperature and mixed with equal quantities of acetone. After dehydration the tissue was placed in this mixture and allowed to soak for 4-6 hours at room temperature. The soaking mixture and tissues were then placed in a 40 C oven for 1-2 hours, after which the tissue was placed in BEEM capsules containing fresh plastic. Sections were cut using glass knives on a Porter Blum M-T 2 ultramicrotome and viewed with a RCA-EMU3 electron microscope.

Quick dips were made by cutting a virus infected leaf and placing the cut end in a drop of potassium phospho tungstate floating in a plastic petri dish. After a short time the cut end of the leaf was removed from the stain, recut, and placed in the stain again. This process was repeated several times. A carbon coated grid was floated on the drop of stain for 1 to 2 minutes, removed and allowed to dry, and then viewed with the electron microscope.

RESULTS

During the course of this study, 223 plants of SDI 6415 were inoculated by both methods and all have been resistant. The donor parent has been indexed. No virus symptoms appeared in the test plants indicating that SDI 6415 is not a symptomless carrier. No virus-like particles were observed with the electron microscope in the donor parent, using thin sections or quick dips.

Cytological examination of SDI 6415 has shown it to have 56 chromosomes (Fig. 1). The average meiotic index for SDI 6415 was 96% which is quite high and corresponds to the observed fertility of 84% as shown in Table 3. There does seem to be some irregularity in chromosome assortment, however. Out of 232 spore quartets observed, 222 were normal, 9 were pentads, and 1 was hexad. No attempt was made to determine how the pentads and the hexad were formed.

Five F_1 plants were examined cytologically. All of them had 21 pairs of chromosomes plus 7 univalents. In Table 3 it can be seen that the seed set on the F_1 was only 4%. Pollen analysis of the normal F_1, SDI 6415 x Minter, revealed 36% of stainable pollen with the reciprocal cross producing 39% of stainable pollen using Lacto-phenol Fuehsin. Thirteen percent of the pollen from the treated F_1 seeds was stainable.

At the time that the material reached the F_3BC_1 and F_4BC_1 generation, the inoculation procedure was switched from the rub method to the blast method. In the last test using the rub method, 148 susceptible check plants were inoculated. Seventy-four of them escaped infection.

Since the adoption of the blast technique there have been 573 susceptible check plants inoculated and only 2 escaped infection. Symptoms varied from faint chlorotic streaks through more intense yellow streaking and mottling to a chlorosis of the entire leaf surface. Affected plants were more or less stunted.

The crosses, levels of irradiation and number of seeds obtained are shown in Table 2. There was 86% seedling survival at 1000 rads of fast neutrons (N^F) and 0% at 2500 rads of N^F. The 1000 rad treatment caused a great amount of sterility. Table 3 shows seed set from selfing and backcrossing parents and hybrids with and without irradiation. Check F_1 plants set seed in 3.9% of main florets compared with 0.1% seed set in F_1 plants from irradiated seed. Table 4 shows an analysis of pollen from certain treated F_1's. There was an average pollen viability as measured by Lacto-phenol Fuehsin of 7%. The range was from 0 to 28% but generally viability was low. The reduction in fertility caused by the irradiation made it necessary to usually backcross, using wheat pollen on the F_1.

Table 5 shows the total number of seeds obtained from selfing or backcrossing F_1 plants. Plants from these seeds were inoculated with WSMV by the rub method and scored for virus reaction. Resistant plants were selected on the basis of similarity to wheat and high fertility. Progenies from these plants were tested against the virus and selection again made on the basis of wheat-like plants of high fertility. The process was continued until the material was advanced to F_3BC_1 and F_4BC_1 generations. Resistant plants were obtained which were very wheat-like and high in fertility.

During selection and progeny testing, two assumptions were made. One was that a single pair of chromosomes carried the resistance factor. The other was that the Agropyron chromosome on which the resistance factor was located did not pair with any of the wheat chromosomes. Therefore, there was very little chance for a natural crossover to occur. If a translocation occurred and if it involved a small enough segment of the Agropyron chromosome carrying resistance, a true breeding line which was high in fertility might be obtained. Such a plant was not found after selfing and selecting for resistance. Apparently, a desirable translocation had not occurred.

A second irradiation treatment would then be necessary to induce a desirable translocation. Before the second treatment, as much of the Agropyron chromatin as possible should be eliminated from the resistant plants. A backcrossing program was begun using as females plants that were wheat-like and resistant to WSMV. F_1 plants without a background of irradiation were also backcrossed to wheat, using wheat as the male parent. The first backcross produced 305 seeds, which were planted and the seedlings inoculated by the blast method. The 305 seeds produced 275 plants, 114 of which were resistant to WSMV. A summary of the test is given in Table 6. In this test there were 43 cultures 15 of which were fully susceptible. From the remaining 28 cultures, 14 resistant plants were selected which did not have a background of irradiation and were more wheat-like than any of the other plants in that group. From the group of plants which had a background of irradiation, 19 were selected as being wheat-like and resistant to WSMV.

There were 320 seeds produced by pollinating these selected plants with wheat pollen. Two hundred and sixty plants resulted, 46 of which were resistant after inoculation by the blast method. A summary of this test is given in Table 7. The resistance was lost in 14 of the 35 cultures. A detailed summary of data on the resistant plants which set seed is given in Table 8.

It was upon certain selected plants that somatic chromosome counts were made. One plant with presumably an added pair of chromosomes carrying resistance to WSMV but with no background of irradiation has been identified. Three plants with a chromosome number of 43 have been identified. Somatic chromosome numbers of the F_1BC_2 plants (Table 8) which have no background of irradiation are generally higher than those which do have an irradiation background.

Two of the resistant plants with 43 chromosomes come from culture 314 and one from culture 318 (Table 8). Plant 1 of culture 314 (Fig. 2) is a wheat-like plant, bearded, and of less than 2% fertility. Plant 4 in culture 314 is the same phenotypically as plant 1 but with a higher percent seed set. The original parents of these plants were SDI 6415 and Lathrop. There were 46 F_1 seeds treated with 1000 rads of fast neutrons. Thirty-two seedlings resulted which were used as females in backcrossing to C6410. Of four F_1BC_1 plants that were tested against WSMV, 1 was resistant and was 64% fertile. This plant was selfed. Of 22 plants in F_2BC_1 that were tested, 14 were resistant. A wheat-like plant was selected and in F_3BC_1 was homozygous for immunity. It should be pointed out that in this test 50% of the susceptible check plants

escaped infection. In F_4BC_1 26 plants were tested and 23 were resistant. This test was inoculated using the blast method. ND321, a spring wheat, was used to pollinate a resistant F_4BC_1 plant which was 8% fertile, producing 4 seeds. These seeds were given the culture number 2028 as shown in Table 6. Plants from these 4 seeds were resistant to WSMV. A resistant plant from culture 2028 was again used as female and pollinated with Hume to produce 5 seeds. Three of the resulting five plants were resistant (Table 7). Two of the plants produced seed and had 43 chromosomes (Table 8). The third resistant plant was completely sterile so no chromosome counts were made.

The third plant with a confirmed chromosome number of 43 (Table 8) is plant 10 in culture number 318 (Fig. 3). It also comes from the resistant plant in F_1BC_1 of (SDI 6415 x Lathrop) x C6410. In F_3BC_1 a plant which was 28% fertile was selfed and 15 plants tested in F_4BC_1, 13 of which were resistant. One of the resistant plants which was 38% fertile was used as female and common wheat backcrossed to it. Culture number 2036 (Table 6) was assigned to the fourteen seeds produced which yielded 10 plants, 4 of which were resistant. Plant number 3 was used as female and ND321 backcrossed to it producing 15 seeds. Thirteen plants in culture 318 resulted as shown in Table 7, four of which were resistant. Plant number 10 was 65% fertile and had 43 chromosomes (Table 8).

Plant 4 in culture 315 (Table 7, Fig. 4) has a confirmed chromosome number of 42 and has almost an identical history to that of the two plants in culture 314. They differ only in which resistant plant was

used as female in F_4BC_1 (Table 6).

Certain selected lines segregating for resistance were examined with the electron microscope. Figure 5 shows cellular tissue from healthy plants. In susceptible plants (Fig. 6, 7 and 9) pinwheel, circular, tube, and bundle inclusions were found. The pinwheel inclusions were similar to those reported by Lee (13). Edwardson (7), Edwardson, Purcifull, and Christie (8) reported that these inclusions were characteristic of viruses 700 to 800 $m\mu$ in length. Very few virus-like particles were seen in thin sections (Fig. 9) but they were quite often observed from quick dips (Fig. 8). No characteristic inclusions or virus-like particles were found in SDI 6415 or in the resistant plants studied.

DISCUSSION

At the beginning of the study it was assumed that the gene for resistance was on one chromosome and that there was no homology between any of the wheat chromosomes and the Agropyron chromosome carrying resistance, precluding crossing over, and that the only way to transfer the resistance was to induce a translocation by irradiation. It is possible that the assumption of one gene on one chromosome is too simple. In Table 8 the resistant plants with no background of irradiation have higher chromosome numbers than those plants which have a background of irradiation. The irradiation may have caused certain minor genes to be lost, although the same level of resistance present in the donor, SDI 6415, seems to be present in the resistant plants studied cytologically. It is also possible that if as much emphasis in selection and crossing had been placed on the nonirradiated material as on the irradiated, these extra chromosomes would have been eliminated.

Many plants of the donor parent have been inoculated, indexed and studied using the electron microscope without detection of virus-like particles. Based on these observations and tests, it is concluded that SDI 6415 is immune to WSMV.

Lines which were segregating for reaction to WSMV were also studied using the electron microscope. Results obtained in the virus susceptible plants were similar to those found in common wheat infected with WSMV by other authors. Circular, pinwheel, tube and bundle inclusions and possibly some virus particles were observed in the

susceptible plants. Resistant plants were free from inclusions as was true of the donor parent. Based on these findings and on the efficiency of the blast method of inoculation the author feels that the same level of resistance which is present in the donor parent is also present in the resistant progeny.

It would be valuable to know the chromosome number of the one resistant plant in F_1BC_1, which produced the two resistant plants in culture 314 and the one plant in culture 315. Since this information is not available one can only speculate as to what happened. There are two possibilities. One is that the irradiation of the F_1 seed had no effect on the chromosome carrying the factor for resistance to WSMV and that we have an addition line in the case of the 43 chromosome plants and a substitution line in the 42 chromosome plant. The possibility of a substitution is quite small. When substitutions have been made by other scientists, it has been necessary to cross a monosomic line of the parent to receive the alien chromosome with a line in which the alien chromosome occurred as an addition monosomic or disomic. Cytological selection was made in the F_1 for plants which contained 20 pairs of wheat chromosomes plus one wheat univalent and one alien univalent. This condition occurred in about 20% of the F_1 plants. These plants were then either selfed or crossed with the addition line and plants selected which had 20 wheat pairs plus one alien chromosome pair. Wienhues (37) has indicated that a chromosome of A. intermedium could act as a substitute for 10 different wheat chromosomes representing all seven homoeologous groups. Such a

substitution would be fairly stable.

The second possibility is that a translocation did occur but that a large enough piece of chromatin was moved over to impart chromosomal instability. A meiotic study of the 42 chromosome plant revealed a meiotic index of 76%, indicating chromosomal instability. A bridge was observed in 4 of the 32 anaphase II cells studied indicating a translocation. There was also a lagging chromosome at AII. Only a few spore mother cells were available for observation. Based then on circumstantial evidence, the latter possibility of a translocation involving a large piece of chromatin seems to be the best explanation. Further cytological evidence on pairing relations is necessary.

After backcrossing once to the irradiated F_1 and selfing 3 or 4 generations while selecting for resistant wheat-like plants high in fertility, a homozygous translocation, if it had occurred, should have been isolated. It was upon such a line that cytological examinations were to be made. Because all of these criteria were not met it was concluded that a desirable translocation had not occurred. With the apparent isolation of an addition line in plants 314-1, 314-4 and 318-10 and a type of translocation in plant 315-4, the best procedure would be to self these plants, select for addition or substitution-like plants from among the selfed progeny and have the material reirradiated.

From irradiation two types of translocations are possible, reciprocal and intercalary, (27). If the gene for resistance lies near the terminal end of a chromosome, then a simple reciprocal

translocation will move the resistance from Agropyron to wheat. The closer the gene for immunity is to the centromere, the more unusable such a translocation becomes because of the possibility of undesirable Agropyron genes being included with the desirable gene. In such a case an intercalary translocation would be the most useful. Such translocations are very rare because they involve two breaks, one on each side of the desirable gene. This piece must then be inserted into a wheat chromosome. Sears (27) suggested using pollen from irradiated plants to increase the selection pressure for an intercalary translocation. It is assumed that the more alien chromatin that is included in the wheat chromosome, the less competitive such pollen is with normal pollen. Advantage is taken of this fact when using pollen from irradiated plants on common wheat.

It is suggested that seed from selfing 42 and 43 chromosome WSMV resistant plants be irradiated and that pollen from the resulting plants be used on a very susceptible spring wheat such as ND 321. Selection among progeny should be made on the basis of chromosome number, normality of chromosome pairing, high fertility, and resistance.

Successful translocations involving irradiation are infrequent. The interchanges are usually gross, arbitrary, and only rarely is a useful one produced. Riley, Chapman, and Johnson (25) have described a method for removing the obstacle to natural recombination between wheat chromosomes of different homoeologous groups and also between wheat and alien chromosomes. The first indication that a single chromosome had control over the diploid type of pairing in wheat was

reported independently and almost simultaneously by two sources. Okamoto (18) crossed a monotelocentric for chromosome 5B with an AADD amphidiploid. Plants lacking telocentric 5B had better pairing and a higher frequency of multivalents than those with 5B. This difference was attributed to a gene or genes for asynapsis on the arm of chromosome 5B. In the other study Riley and Chapman (23), using nulli haploids (20 chromosomes) in the variety Holdfast deficient for 5B, found a much higher frequency of multivalent formation than in the euhaploids. The conclusion was that chromosome 5B carried a gene or genes effective in single dose which restricted intergenomic pairing. Riley (23) found that there was a gene in Triticum speltoidies that was able to suppress the genetic effect of 5B in 27 and 28 chromosome hybrids of monosomic 5B and T. speltoidies.

There exists two possibilities for the suppression of the genetic effect of 5B. One is to cross the addition line to the nullisomic for 5B. Nulli 5B is male sterile and it would thus be necessary to use it as female. The male sterility may also be carried over to the hybrids making them hard to maintain (21). Riley and Kempanna (24) found that extra doses of the homoeologous chromosome 5D can be introduced to overcome the sterility barrier. If the addition monosome resistant to WSMV was crossed with Chinese Spring nulli 5B-tetra 5D, the resistant F_1 plants would have 43 chromosomes. These plants could then be selfed or backcrossed to nulli 5B-tetra 5D. Progeny would be inoculated and further study restricted to the resistant plants. At this point cytological examination would be necessary. There would be plants which are resistant and still have

chromosome 5B. These plants would be similar to the F_1 in which case pairing would be normal (24). In plants which lacked 5B there would be a higher level of multivalent formation. It is these plants which should be selfed several generations while preventing outcrossing to allow for pairing between the alien chromosome and any wheat chromosome. At the end of the several generations of selfing, chromosome 5B should be restored. Selection would then be made for wheat-like resistant plants high in fertility.

The other possibility is to cross the alien addition line with T. speltoidies. Riley et al. (25) used this method in transferring yellow rust resistance from T. comosa (2n=14) to wheat. He crossed a 43 chromosome plant which had all the wheat chromosomes plus the one chromosome from T. comosa carrying yellow rust resistance with T. speltoidies. The F_1 was backcrossed to wheat and the F_1BC_1 progeny tested for reaction to yellow rust. One self sterile resistant plant with 38 chromosomes was backcrossed to wheat again. Plants which resulted were again tested against yellow rust and chromosomes of resistant plants counted. A 41 chromosome plant was backcrossed to wheat and a resistant 42 chromosome plant selected. Meiosis was normal and the phenotype of the wheat parent was fully recovered. The variety Compair, with the yellow rust resistance of T. comosa, was released.

Significant progress has been made thus far in transferring the WSMV immunity of SDI 6415 to wheat. An aggressive effort using the resistant material developed thus far and methods described should

overcome the problem of susceptibility to WSMV in common wheat varieties.

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Table 1. Reaction of T-A lines mechanically inoculated with WSMV

T-A line	Reaction to WSMV*	Growth habit**
WG 57374	5	S
WG 57375	5	S
WG 57376	5	S
WG 58401	5	S
WG 58409	5	S
Tc^6-Rescue-*A. elongatum*	5	S
L 709	3	W
L 710, Hy. 59	4	W
L 609	5	W
L 608	4	W
L 690	dead	W
L 691 Ta 25	1	W
Sac. 4191 (Sebesta 46)	3	W
Sac. 4212 (Sebesta 47)	3-4	W
Sac. 4212 sel. 1 Sebesta 47-1	2	W
Sac. 4251 Sebesta 52	4	W
SS 734 Sebesta 69	2	W
SS 734 sel. 1 Sebesta 69-1	2	W
SS 792 Sebesta 75-1	4	W
SS 700 Sebesta 64	3-4	W
Sac. 4177 Sebesta 45	2	W

* 1 = resistant 5 = susceptible

** S = spring habit W = winter habit

Table 2. Doses of fast neutrons and seedling survival from F_1 seeds

Crosses	2500 rads		1000 rads	
	seeds	seedlings	seeds	seedlings
Lee x SDI 6415	38	0	--	--
SDI 6415 x Lee	10	0	17	15
SDI 6415 x Crim	36	0	--	--
SDI 6415 x Reward	--	-	25	23
SDI 6415 x Marquis	47	0	42	37
SDI 6415 x Selkirk	57	0	--	--
SDI 6415 x Pembina	111	0	141	118
SDI 6415 x Lathrop	--	-	46	32
SDI 6415 x ND 373	--	-	30	25
SDI 6415 x ND 260	53	0	53	47
SDI 6415 x ND 364	--	-	38	38
SDI 6415 x Chris	--	-	31	28
SDI 6415 x II 55-11	43	0	8	8
SDI 6415 x II 55-12	37	0	--	--
SDI 6415 x Minter	--	-	28	24
SDI 6415 x Yogo	28	0	--	--
SDI 6415 x Lancer	4	0	--	--
SDI 6415 x NB61954	13	0	--	--
SDI 6415 x Quivira hybrid	--	-	15	12
Totals	477	0	474	407

Table 3. A measure of relative fertilities of parents and hybrids with or without irradiation of the seed from which the plants were grown.

Parent or cross	Previous treatment	Operation	Main florets	Seeds	% Seed set
SDI 6415 (P_1)	check	Selfed	532	445	83.6
Lee (P_2)	check	Selfed	24	24	100.0
F_1	check	Selfed	1054	41	3.9
F_1 x wheat	check	Backcrossed	218	42	19.3
Wheat x F_1	check	Backcrossed	212	11	5.2
P_1	N^{F*}	Selfed	4220	1849	43.8
F_1	N^{F*}	Selfed	1961	2	0.1
$P_1(N^F)$ x wheat	N^{F*}	Backcrossed	246	66	26.8
Wheat x $P_1(N^F)$	N^{F*}	Backcrossed	725	65	9.0
$F_1(N^F)$ x wheat	N^{F*}	Backcrossed	4422	166	3.8
Wheat x $F_1(N^F)$	N^{F*}	Backcrossed	214	6**	2.8

* 1000 rads of fast neutrons on the seed

** These may have been selfs

Table 4. Stainability of pollen of selected F_1 plants grown from seed treated with 1000 rads of fast neutrons.

Crosses	Non-staining	Staining	Percent viable pollen
SDI 6415 x Pembina	35	1	3
SDI 6415 x ND 260	10	1	10
SDI 6415 x Marquis	25	7	28
SDI 6415 x Chris	9	1	11
SDI 6415 x ND 364	22	1	5
SDI 6415 x Reward	20	0	0
SDI 6415 x Lee	20	0	0
SDI 6415 x ND 373	11	1	9
SDI 6415 x II 55-11	22	1	5
	174	13	6.9

Table 5. Total seeds obtained from selfing and backcrossing irradiated and nonirradiated plants.

Parents	Plant generation	Treatment	Seed no.
(SDI 6415 x wheat) x wheat	F_1BC_1	None	46
(SDI 6415 x wheat) x wheat	F_1BC_1	1000 rads on F_1 seed	86
SDI 6415 x wheat	F_2	None	36
SDI 6415 x wheat	F_2	1000 rads on F_1 seed	19
Total			187

Table 6. Tests of progeny from a single backcross of the F_1 (SDI 6415 x wheat) and immune segregates to wheat.

Culture No.	Pedigree	WSMV Reaction Res.	Susc.
2001	F_1BC_1	7	1(dead)
2002	F_1BC_1	7	2
2003	F_1BC_1	10	6
2004	F_1BC_1	1	7
2005	F_1BC_1	2	0
2006	F_2 sel. x wheat*	20	14
2007	F_2 sel. x wheat	--	--
2008	F_2 sel. x wheat	0	1
2009	F_2 sel. x wheat	--	--
2010	F_2 sel. x wheat	1	0
2011	F_2 sel. x wheat	0	1
2012	F_4 sel. from N^F F_1 x wheat**	5	5
2013	F_3 sel. from N^F F_1 x wheat	2	0
2014	F_3 sel. from N^F F_1 x wheat	10	0
2015	F_3BC_1 sel. from N^F F_1 x wheat	1	0
2016	F_3BC_1 sel. from N^F F_1 x wheat	0	7
2017	F_3BC_1 sel. from N^F F_1 x wheat	0	1
2018	F_3BC_1 sel. from N^F F_1 x wheat	0	11
2019	F_3BC_1 sel. from N^F F_1 x wheat	1	14
2020	F_3BC_1 sel. from N^F F_1 x wheat	0	6
2021	F_3BC_1 sel. from N^F F_1 x wheat	0	5

Table 6. Continued

Culture No.	Pedigree	WSMV Reaction Res.	Susc.
2022	F_4BC_1 sel. from N^F F_1 x wheat	10	7
2023	F_4BC_1 sel. from N^F F_1 x wheat	5	0
2024	F_3BC_1 sel. from N^F F_1 x wheat	2	0
2025	F_4BC_1 sel. from N^F F_1 x wheat	2	1
2026	F_4BC_1 sel. from N^F F_1 x wheat	6	0
2027	F_4BC_1 sel. from N^F F_1 x wheat	2	14
2028	F_4BC_1 sel. from N^F F_1 x wheat	4	0
2029	F_4BC_1 sel. from N^F F_1 x wheat	1	0
2030	F_4BC_1 sel. from N^F F_1 x wheat	--	--
2031	F_4BC_1 sel. from N^F F_1 x wheat	5	3
2032	F_4BC_1 sel. from N^F F_1 x wheat	1	8
2033	F_3BC_1 sel. from N^F F_1 x wheat	0	1
2034	F_3BC_1 sel. from N^F F_1 x wheat	2	4
2035	F_3BC_1 sel. from N^F F_1 x wheat	1	0
2036	F_4BC_1 sel. from N^F F_1 x wheat	4	6
2037	F_4 sel. from (N^F donor x wheat) x wheat	0	10
2038	F_4 sel. from (N^F donor x wheat) x wheat	0	4
2039	F_4 sel. from (N^F donor x wheat) x wheat	0	2
2040	F_4 sel. from (N^F donor x wheat) x wheat	0	1
2041	F_3BC_1 sel. from N^F F_1 x wheat	0	9
2042	F_4BC_1 sel. from N^F F_1 x wheat	0	1

Table 6. Continued

Culture No.	Pedigree	WSMV Reaction Res.	Susc.
2043	F_4BC_1 sel. from N^F F_1 x wheat	1	5
2044	ND 321	0	5
2045	Hume	1	4
	Total	114	166

* selected on the basis of being wheat-like, high in fertility and resistant to WSMV.

** 1000 rads of fast neutrons N^F on seed

Table 7. Progeny tests of plants after the second backcross to selected immune plants.

Culture No.	Source		Pedigree		WSMV Reaction	
	Culture	plant	no irradiation	1000 r on F_1 seed	Res.	Susc.
286	2001	1	F_1BC_1 sel. x wheat		0	22
287	2001	2	F_1BC_1 sel. x wheat		-	--
288	2001	3	F_1BC_1 sel. x wheat		0	4
289	2001	4	F_1BC_1 sel. x wheat		1	0
290	2002	1	F_1BC_1 sel. x wheat		5	9
291	2002	4	F_1BC_1 sel. x wheat		1	9
292	2002	5	F_1BC_1 sel. x wheat		4	4
293	2003	1	F_1BC_1 sel. x wheat		0	6
294	2003	2	F_1BC_1 sel. x wheat		0	2
295	2005	1	F_1BC_1 sel. x wheat		0	1
296	2005	1	F_1BC_1 sel. x wheat		-	--
297	2006	1	(F_2 sel. x wheat) x wheat		3	0
298	2006	2	(F_2 sel. x wheat) x wheat		2	5
299	2006	3	(F_2 sel. x wheat) x wheat		1	3

Table 7. Continued

Culture No.	Source Culture	plant	Pedigree no irradiation	1000 r on F_1 seed	WSMV Reaction Res.	Susc.
300	2010	1	(F_2 sel. x wheat) x wheat		1	0
301	2012	1		(F_4 sel. x wheat) x wheat	0	11
302	2012	1		(F_4 sel. x wheat) x wheat	0	8
303	2012	2		(F_4 sel. x wheat) x wheat	5	15
304	2013	1		(F_3 sel. x wheat) x wheat	1	0
305	2013	1		(F_3 sel. x wheat) x wheat	0	4
306	2014	1		(F_3 sel. x wheat) x wheat	1	5
307	2014	2		(F_3 sel. x wheat) x wheat	3	10
308	2019	1		(F_3BC_1 sel. x wheat) x wheat	0	19
309	2019	2		(F_3BC_1 sel. x wheat) x wheat	0	18
310	2019	3		(F_3BC_1 sel. x wheat) x wheat	0	2
311	2022	1		(F_4BC_1 sel. x wheat) x wheat	0	6
312	2022	2		(F_4BC_1 sel. x wheat) x wheat	1	6
313	2022	3		(F_4BC_1 sel. x wheat) x wheat	0	2

Table 7. Continued

Culture No.	Source: Culture	Source: plant	Pedigree: no irradiation	Pedigree: 1000 r on F_1 seed	WSMV Reaction: Res.	WSMV Reaction: Susc.
314	2028	1		(F_4BC_1 sel. x wheat) x wheat	3	2
315	2029	1		(F_4BC_1 sel. x wheat) x wheat	2	6
316	2035	1		(F_3BC_1 sel. x wheat) x wheat	-	--
317	2036	1		(F_4BC_1 sel. x wheat) x wheat	3	9
318	2036	3		(F_4BC_1 sel. x wheat) x wheat	4	9
319	2036	4		(F_4BC_1 sel. x wheat) x wheat	2	10
320	2043	1		(F_4BC_1 sel. x wheat) x wheat	3	7
321	2043	2		(F_4BC_1 sel. x wheat) x wheat	-	--
			Total		46	214

Table 8. Characteristics of immune plants after two backcrosses to resistant plants.

Culture	Plant	Percent* seed set	Chromosome number	Plant type
290	2	2	45**	Resembles SDI 6415, late
290	11	2	--	Resembles SDI 6415
292	1	80	45	Resembles SDI 6415
292	7	40	--	Resembles SDI 6415, late
299	2	12	45	Resembles SDI 6415
303	3	50	--	Wheat-like, beardless
303	7	18	44**	Wheat-like, beardless
303	13	2	43	Wheat-like, beardless
303	14	18	--	Wheat-like, bearded
303	20	13	43	Wheat-like, beardless
306	4	21	--	Wheat-like, bearded
307	7	85	--	Wheat-like, bearded
307	9	64	--	Wheat-like, bearded, short
312	3	46	--	Wheat-like, bearded
314	1	2	43**	Wheat-like, bearded
314	4	41	43**	Wheat-like, bearded
315	1	9	--	Wheat-like, bearded
315	4	61	42**	Wheat-like, bearded
317	2	72	42	Wheat-like, bearded
317	10	2	--	Short weak plant which might be susc.
318	7	53	--	Faint virus symptom appeared later
318	8	31	--	Wheat-like, bearded
318	9	74	--	Wheat-like, bearded
318	10	65	43**	Wheat-like, bearded
319	4	68	--	Wheat-like, bearded
319	7	80	--	Wheat-like, bearded

Table 8. Continued

Culture	Plant	Percent* seed set	Chromosome number	Plant type
320	2	55	--	Wheat-like, bearded
320	8	45	--	Wheat-like, bearded
320	9	18	44	Wheat-like, many tillers

* determined by counting seeds in the main florets

** these chromosome numbers have been confirmed

Fig. 1. Fifty-six somatic chromosomes of SDI 6415, x 2530.

Fig.2

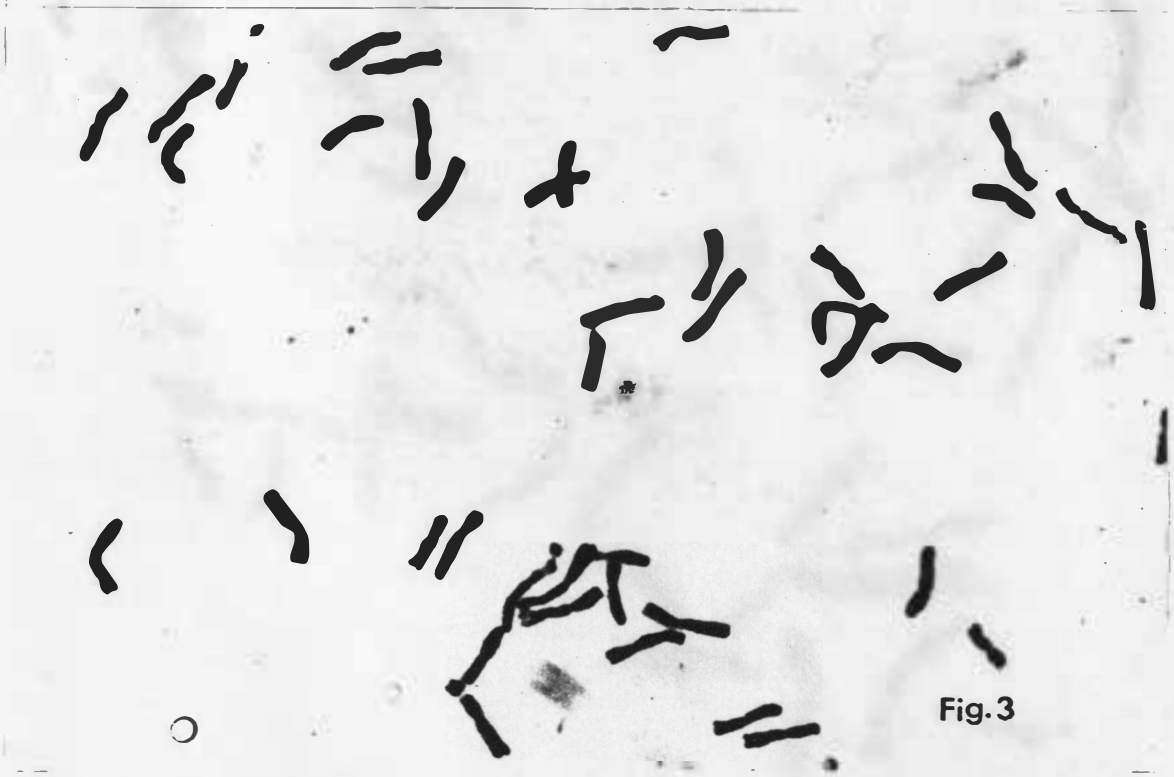


Fig.3

Fig. 2. Somatic chromosomes (43) of Culture 314, plant 1, x 1690.

Fig. 3. Somatic chromosomes (43) of Culture 318 plant 10, x 1480.

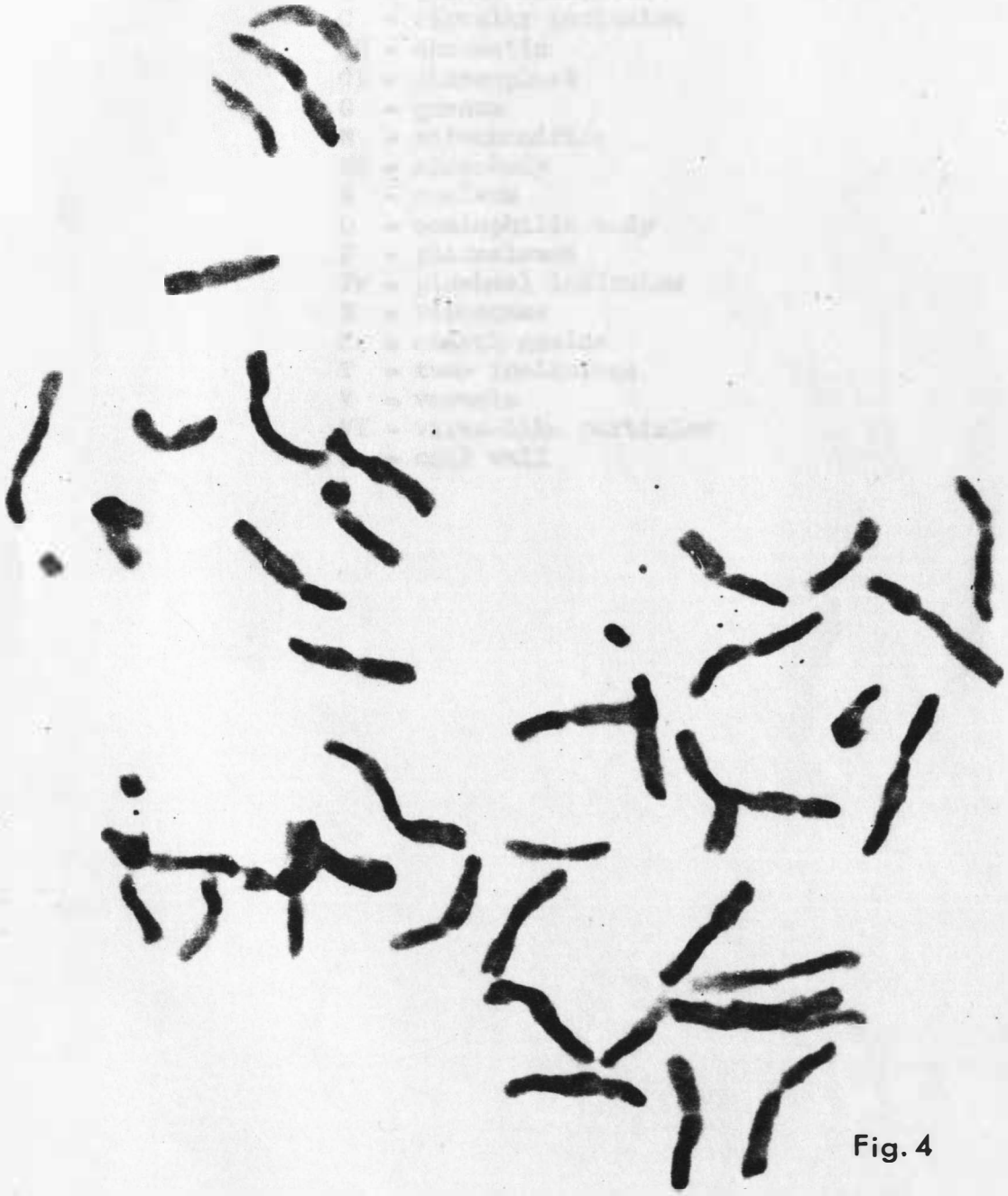


Fig. 4. Somatic chromosomes (42) of Culture 315, plant 4, x 1960.

Key to Labeling of Electron Micrographs

B = bundle inclusion
C = circular inclusion
CH = chromatin
CL = chloroplast
G = granum
M = mitochondrion
MB = microbody
N = nucleus
O = osmiophilic body
P = plasmalemma
PW = pinwheel inclusion
R = ribosomes
S = starch grains
T = tube inclusions
V = vacuole
VI - virus-like particles
W = cell wall

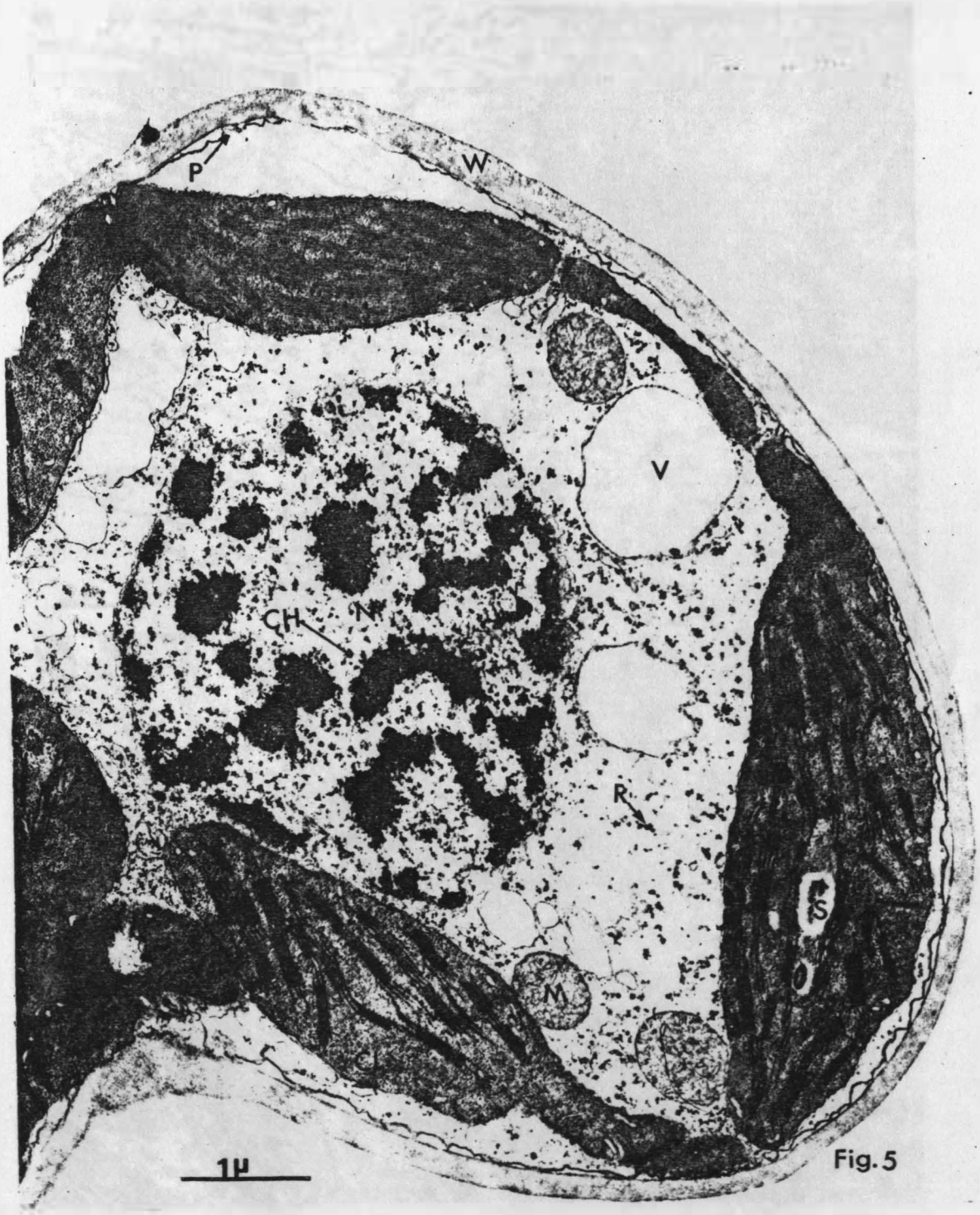
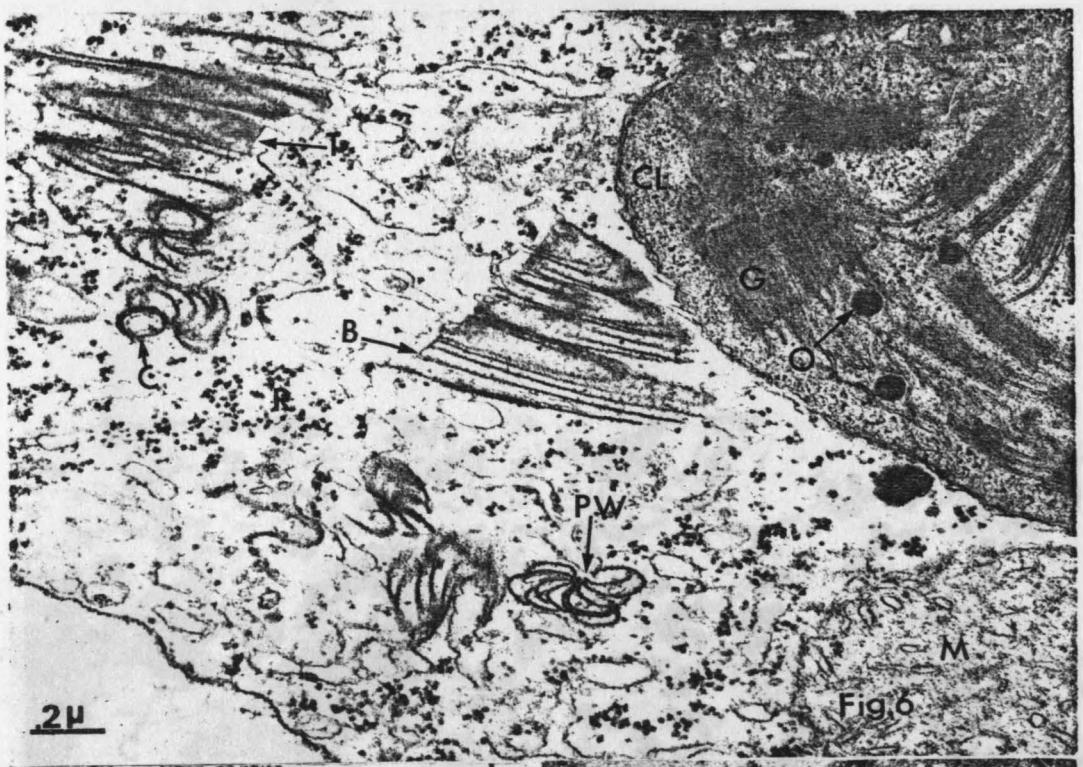
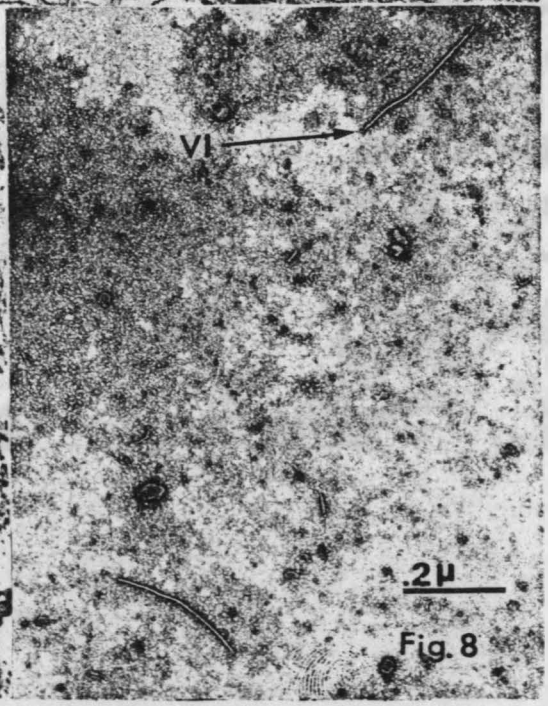


Fig. 5. Electron micrograph of tissue from a symptomless plant within a culture which was segregating. There are no inclusions or virus-like particles present.





Electron micrograph of virus infected tissue showing 4 types of inclusions in one cell (Fig. 6.), two types of inclusions in another cell (Fig. 7.), and virus-like particles obtained by quick dips from virus infected plants (Fig. 8.).

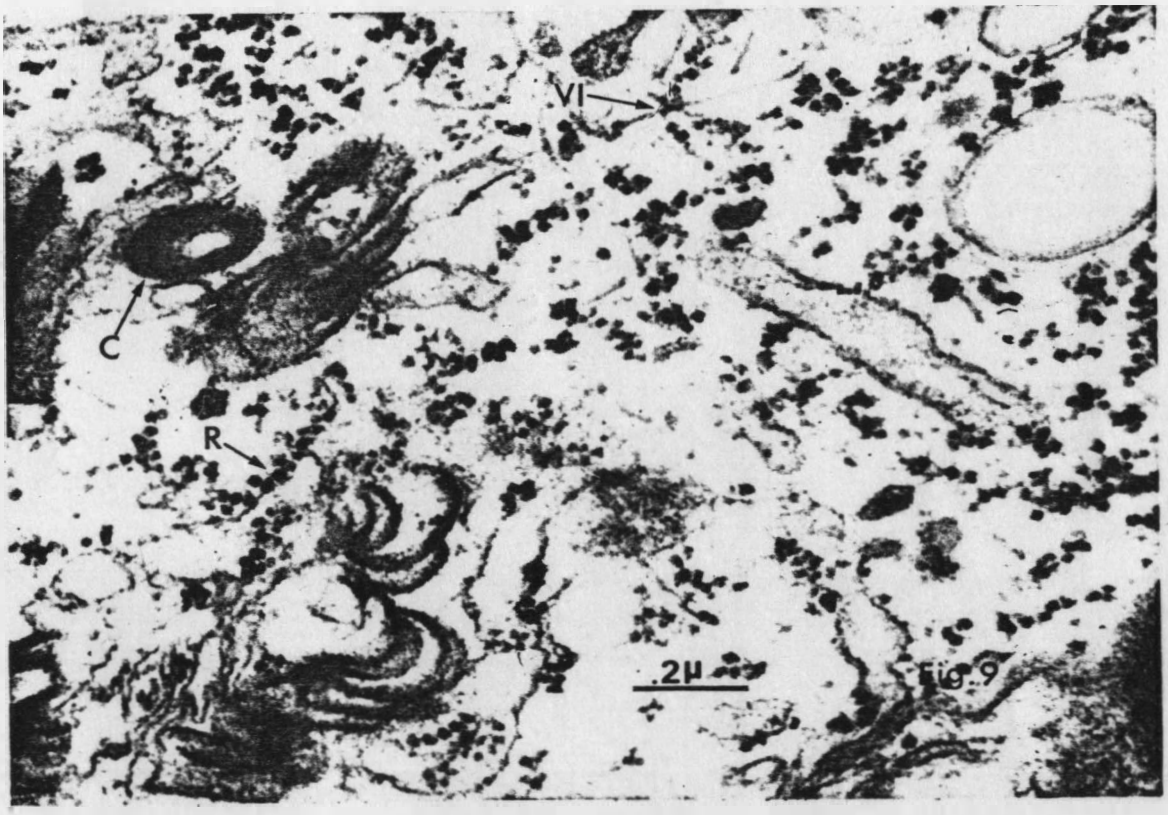


Fig. 9. Electron micrograph of virus infected tissue showing virus-like particles and circular inclusions.